



Application Serial Number 10/086,177

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Re: Application Serial Number 10/086,177

Inventors: TUDAN et al.

Filed: 26 February 2002

Title: CXCR4 AGONIST TREATMENT OF HEMATOPOIETIC CELLS

Declaration Under Rule 1.132

I, Dr. Ahmed Merzouk, a resident of Vancouver, British Columbia, Canada, and a scientist currently employed by Chemokine Therapeutics Corp. declare as follows:

- 1) All statements herein of my knowledge are true and all statements herein on information and belief are believed to be true.
- 2) I earned a Ph.D. in November 1993 from Paris-Sud University, France, in Organic Chemistry. I am presently employed by Chemokine Therapeutics Corp. in Vancouver, British Columbia, Canada, as the Senior Peptide Chemist. Amongst other things, I am responsible for studying protein receptor interactions.
- 3) I understand that I am listed as a co-inventor of the invention described and claimed in the aforementioned United States Patent Application.
- 4) I understand that the following claim is currently pending in the foregoing application:

33. A CXCR4 agonist wherein the agonist is H-[Ala⁹-Phc¹¹]-SDF-(1-14)-LINKER-cyclo(Lys⁵⁶-Glu⁶⁰)-SDF-(55-67)-NH₂, having the sequence

KPVSLSYRAPFRFF-[LINKER]-LKWIQEYLEKALN-NH₂
(SEQ ID NOS:208-210)

wherein the LINKER is of the formula G₁₋₄ (SEQ ID NO:213) or (CH₂)₁₋₂₀.

- 5) I understand that the Examiner has expression the following reservations, among others, with respect to the foregoing claim, in an Advisory Action dated 28 August 2006:

A large quantity of experimentation would be required of the skilled artisan to generate an agonist peptide with the large number of linkers recited in the claim, other than a linker with 4 glycine residues...one skilled in the art would not be able to predict the proper structural orientation and function of SDF analogs with different types and numbers of linkers.

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- 6) I have reviewed the patent application, pending claims and the examiner's concerns about the linker between the termini of the recited peptides. As I understand the Examiner's concerns, she believes that the length and make up of the linker is critical and any substitutions from the four glycines currently being used would lead to very unpredictable activity.
- 7) In recent studies, we have demonstrated that agonists of CXCR4 having a wide variety of 4 amino acid linkers (combinations of Glycine and Arginine, or Glycine and Lysine) have substantial biological and CXCR4 binding activity. The linkers are as follows:

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Lys-Gly-Gly-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Lys-Gly-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Gly-Lys-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Gly-Gly-Lys-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Lys-Lys-Gly-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Lys-Lys-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Gly-Lys-Lys-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Lys-Gly-Gly-Lys-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Lys-Gly-Lys-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Lys-Gly-Lys-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Lys-Lys-Lys-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Lys-Lys-Lys-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Lys-Gly-Lys-Lys-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Lys-Lys-Gly-Lys-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Lys-Lys-Lys-Lys-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Arg-Gly-Gly-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Arg-Gly-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

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Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Gly-Arg-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Gly-Gly-Arg-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Arg-Arg-Gly-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Arg-Arg-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Gly-Arg-Arg-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Arg-Gly-Gly-Arg-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Arg-Gly-Arg-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Arg-Gly-Arg-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Arg-Arg-Arg-Gly-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Gly-Arg-Arg-Arg-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Arg-Arg-Gly-Arg-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

Lys-Pro-Val-Ser-Leu-Ser-Tyr-Arg-Ala-Pro-Phe-Arg-Phe-Phe-Arg-Arg-Arg-Arg-Leu-
Lys-Trp-Ile-Gln-Glu-Tyr-Ileu-Glu-Lys-Ala-Leu-Asn

- 8) The CXCR4 binding efficacy of the foregoing peptide compounds was demonstrated by their ability to compete with SDF-1 for binding to CXCR4 receptors on the surface of SUPT1 cells. For these assays, SUP-T1 cells (ATCC), a human lymphoid cell line, were used at a concentration of 5×10^6 cells/ml. The Durapore membrane of Millipore MultiScreen 96-well plates was blocked with a PVP/Tween-based blocking buffer before use. RPMI-based binding buffer, SDF-1 (0-400 nM) or peptide analogue (0-400 μ M), 0.02 nM [125]-SDF-1 (Amersham) and SUP-T1 cells were added to wells. Plates were incubated at 4°C with shaking for 2h, followed by triplicate washes with PBS. Bound [125]-SDF-1 was counted using a CliniGamma gamma counter (LKB Wallac). Experiments were performed in triplicate. Homologous (SDF-1) and heterologous (CTCE-9908) competition curves were fitted with Graphpad Prism v4.0 after subtracting non-specific binding to both filters and cells. The K_i for the compounds was tested, all of which illustrate significant CXCR4 binding.
- 9) The CXCR4 receptor activation efficacy of the foregoing peptides was demonstrated by their ability to mobilize intracellular calcium in SUPT1 cells. For these assays, SUP-T1 cells were plated on the day of the assay at 1.2×10^5 cells per well of 96-well black wall/clear bottom plates coated with poly-D-lysine (Becton Dickinson) and loaded with the fluorescent calcium

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indicator FLIPR Calcium 3 assay kit component A (Molecular Probes) for 1hr at 37°C. The intracellular calcium mobilization in response to the appropriate analogue was measured at 37°C by monitoring the fluorescence as a function of time simultaneously in all the wells using the Flexstation Fluorometric Imaging Plate Reader (Molecular Devices). The EC50 of the peptides was determined, indicated that all showed CXCR4 receptor activation activity.

- 10) Based upon my experience in this field of over 10 years, as confirmed by the foregoing results, I can confidently state that the claimed linkers of the formula G_{1-4} or $(CH_2)_{1-20}$ function as spacers that serve the purpose of positioning the two termini for binding to CXCR4. An alkyl bridge or a glycine bridge are readily interchangeable, and while 4 angstroms of space is desired, some activity would be expected using compounds having linkers of the formula G_{1-4} or $(CH_2)_{1-20}$. Based on the above experimental results and the understood nature of linkers in circumstances such as these, there is no objective, scientific reason to believe that the chemical nature of the linker is critical to the functionality of the peptides of this invention. Moreover the preference for amino acid linkers over an alkyl bridge is one of convenience. The amino acid linker is added to the gene and is expressed with the active portions of the proteins in a single step while attaching alkyl bridges to two peptides requires additional work.
- 11) I acknowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and may jeopardise the validity of the patent application and any patent issuing thereon.



Dr. Ahmed Merzouk, 11 October 2006

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